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Chemical Parameters Analysis of Diverse Casing Substrates Across *Agaricus bisporus* (Lange) Imbach Development under Natural Bamboo Hut Conditions

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Abstract

The present study was conducted from September, 2019 to April, 2020 at College of Horticulture and Forestry, Neri, Hamirpur, Himachal Pradesh, India with an aim to examine the chemical parameters of various casing substrates and their changes throughout the crop development stages in *Agaricus bisporus* (Lange) Imbach production, under natural bamboo hut conditions. Chemical parameters of seven casing media viz., sawdust, spent compost (aged 2 years), coconut coir pith, farmyard manure (aged 2 years), vermicompost, and local soil were evaluated, amongst which, independent of the days after casing, local soil recorded the highest mean pH of 7.48, while the lowest mean pH of 6.17 was recorded in two-year-old spent compost. For electrical conductivity (EC), local soil recorded the highest mean value of 1150.04 µS and coconut coir pith recorded the lowest at 641.67 µS. In the commercial trial, irrespective of the days after casing, vermicompost-leached alone recorded the highest mean pH of 7.43, whereas the lowest mean pH of 6.30 was recorded in the control treatment (farmyard manure+local soil). Regarding EC, vermicompost-leached alone recorded the highest mean value of 1162.37 µS, while coconut coir pith recorded the lowest at 658.56 µS. Both pH and EC were tracked for up to 56 days after casing and the findings revealed that the pH of all casing substrates decreased as the crop progressed, while EC consistently increased over time.

Keywords: *Agaricus bisporus*, pH, electrical conductivity, casing, bamboo hut

1. Introduction

Agaricus bisporus (Lange) Imbach, from the Agaricaceae family, is cultivated in over 70 countries across all continents except Antarctica, making it the most widely grown and popular edible mushroom globally and the top species among cultivated mushrooms (Royse et al., 2017; Usman et al., 2021). It accounts for about 31% of global mushroom production. China is the largest producer, with an annual output of about 2.3 mt in 2019. In India, it makes up 85% of the total mushroom production (Sharma et al., 2017; Singh et al., 2021) and is even exported to East Asian countries in high volume (Gao et al., 2023).

A. bisporus, commonly known as button mushroom, is not only a valuable food source but also a rich source of several important bioactive compounds that contribute to human health (Khan et al., 2015; Usman et al., 2021). Over the past few years, numerous bioactive compounds with nutritional value have been isolated from *A. bisporus* (Ruthes et al., 2013). It is low in calories but high in quality protein, dietary fiber, vitamins B and D, and various essential minerals (Dhawan and Chakraborty, 2024). Moreover, it is rich in phenolic compounds and possesses significant antioxidant activity (Pleșoianu and Nour, 2022).In addition to its nutritional benefits, *A. bisporus* plays a role in the cosmetics industry due to certain constituents that help address skin issues and enhance facial beauty (Shbeeb et al., 2019). Fresh button mushrooms are available in various colors (white, off-white, brown), sizes (from small with a 15 mm cap diameter to large with caps over 50 mm), and maturation levels (from fully closed to fully opened caps) (Baars et al., 2020).

The nutritional value of commercially grown mushrooms is heavily influenced by the chemical makeup of their growing substrate (Kumar et al., 2022; Abou Fayssal et al., 2020; Abou Fayssal et al., 2021). Casing layer plays a crucial role in mushroom cultivation, providing several essential functions such as physical support for the carpophores, acting as a water reservoir, facilitating nutrient exchange, regulating osmotic pressure and creating an aerated environment conducive to efficient metabolism. It also stimulates fructification, allows for gas exchanges, and offers zones for ion exchange (Kacprzak et al., 2017). To fulfil these roles effectively, the casing material must possess suitable physicochemical and biological properties that encourage pinhead formation (Sajyan et al., 2021). Ideally, a casing soil should have a water holding capacity above 45%, porosity over 50%, a pH of around 7.8, and an electrical conductivity (EC) below 1.6 mhos (Cunha Zied et al., 2011). As the mushroom crop progresses, the accumulation of ions and soluble salts in the casing layer leads to an increase in electrical conductivity (EC). This rise in EC is mainly attributed to the accumulation of potassium, calcium, chloride, sodium, and sulfate ions in the casing material during the cropping period (Shandilya and Hayes, 1987; Shandilya, 1989). Loam soil, a surface layer organic soil, is the most commonly used casing material on a commercial scale in India. However, various agro-industrial residues, such as composted vine shoots, spent mushroom substrate, coconut fiber pith, and waste paper, have been tested as alternatives or additives to loam soil (Mami et al., 2013; Adibian and Mami, 2015).

Therefore, the present study was conducted with an aim to examine the chemical parameters of various casing substrates and their changes throughout the crop development stages in *Agaricus bisporus* (Lange) Imbach production under natural bamboo hut conditions.

2. Materials and Methods

2.1. Procurement of different casing substrates

The experiments were conducted from September, 2019 to April, 2020 at College of Horticulture and Forestry, Neri, Hamirpur, Himachal Pradesh, India (31.6968217˚N, 76.4658737˚E), under natural bamboo hut conditions. Seven casing substrates were utilized in the study, namely, sawdust, spent compost (aged 2 years), coconut coir pith, farmyard manure (aged 2 years), vermicompost, and local soil. Additionally, a standard check consisting of farmyard manure and local soil in a 3:1 volume ratio was included. All of these casing substrates were locally sourced from the Neri and Hamirpur regions.

The obtained sawdust and vermicompost were subjected to a fourteen-day leaching process within a large jute sack before utilization in the experiments. Water was introduced into the sacks through the open tops using a mug until water droplets began to emerge from the bottom of the sack. This leaching method aimed to remove excess nutrients from these materials.

2.2. Preparation of spawn

After washing and boiling wheat grains, they were combined with calcium carbonate and gypsum, filled into glass bottles, and autoclaved at 22.5 lb sq^{-1} inch pressure for 2 hours and 20 minutes. Following inoculation with *A. bisporus* pure culture mycelium, the bottles were incubated at 25°C, creating the master culture. This master culture, stored in the refrigerator, was utilized to multiply spawn for experiments, with fresh spawn from fully grown cultures used for spawning in various experiments.

2.3. Determination of chemical parameters of casing materials

To ascertain the chemical characteristics of casing materials and understand the dynamics of diverse casing substrates throughout the crop development in *Agaricus bisporus* production, two experiments were undertaken. The initial or preliminary trial, followed by a subsequent commercial trial. In the latter trial, different casing combinations were employed, utilizing sawdust with the top-performing four individuals selected based on yield in the preliminary trial in 1:1 ratio. These individuals were then compared against their respective treatments and a standard check, all implemented in 10 kg capacity growing compost bags. Both experiments were conducted using a completely randomized design, with each treatment replicated three times.

Prior to commencing both the experiments and casing of the mushroom bags (Day 0), pH and electrical conductivity (EC), two chemical parameters, were evaluated for the seven different casing substrates. For pH determination, 10 g of each casing sample was individually weighed, mixed with 100 ml of distilled water, and shaken well. After overnight incubation at room temperature, the pH of each suspension was measured using a pH meter (Model No. "LMPH-10"). For EC assessment, 10 g of casing samples were individually weighed, combined with 100 ml of distilled water, and shaken thoroughly. After overnight incubation at room temperature, the conductivity of each mixture was measured using a conductivity meter (Model No. "LMCM-20").

In both the preliminary and commercial research trials, variations in pH values of different casing substrates were monitored during cropping. Samples from each replication of various treatments in all three experiments were collected, weighed, and their pH recorded at intervals: 0, +7, +14, +21, +28, +35, +42, and +56 days after casing. Additionally, for electrical conductivity determination during cropping, samples from different treatments were collected on the same days, weighed, and analyzed as previously described.

2.4. Spawning and casing of the growing bags

The compost was meticulously spawned at a rate of 0.75%, and the resulting compost bags were tightly sealed to preserve moisture. Positioned on bamboo hut shelves, relative humidity was upheld by daily water spraying. Typically, ready for casing in 15–17 days, the room temperature for spawn run was maintained by heating as needed.

Before casing the mushroom bags, all test casing materials underwent chemical treatment with Formalin (12.5%)+Bavistin (0.1%)+Nuvan (0.1%). The casing moisture level was adjusted to 60%. In the natural bamboo hut, efforts were made to sustain favorable conditions of 14–19°C temperature and 80–90% relative humidity until the cropping concluded. Humidity maintenance involved spraying water on hut walls, floors, and cropping bags as needed.

3. Results and Discussion

3.1. Analysis of chemical parameters of different casing substrates

3.1.1. Preliminary trial

The chemical parameters, namely pH (hydrogen ion concentration) and electrical conductivity, were analyzed for various casing substrates both independently and in optimal treatment combinations during both the preliminary and commercial experiments. The recorded data is presented in Tables 1 and 2.

Table 1: Analysis of chemical parameters of different casing substrates

Casing medium	pH	Electrical conductivity (µS)
Sawdust-leached	7.29	688.00
Farmyard manure-2 years old	7.14	593.67
Vermicompost-leached	7.53	762.33
Local soil	7.80	762.33
Coconut coir pith	6.73	238.00
Spent compost-2 years old	6.45	498.67
Control (farmyard manure+local soil)	6.50	462.67
CD (*p*=0.05)	0.14	11.71
SE(d)	0.06	5.41

The mean minimum pH (6.45) was observed in spent compost aged 2 years, which was statistically comparable to the control treatment (6.50), consisting of farmyard manure and local soil in a 1:1 ratio (v/v). Following closely was coconut coir pith with a pH of 6.73. Conversely, the mean maximum pH (7.80) was significantly noted in the local soil casing substrate, followed by vermicompost-leached with a pH of 7.53.

Regarding electrical conductivity of the casing substrates, the average minimum value (238.00 µS) was recorded in coconut coir pith, significantly followed by the control treatment (462.67 µS), spent compost aged 2 years (498.67 µS), and farmyard manure aged 2 years (593.67 µS). In contrast, the mean maximum electrical conductivity (762.33 µS) was significantly recorded in vermicompost-leached and local soil, followed by sawdust leached with a conductivity of 688.00 µS.

3.1.2. Commercial trial

In the final commercial trial, the pH values of casing substrates varied (Table 2), with the lowest mean pH (6.52) observed in the control group. Notably, sawdust-leached+spent compost, sawdust-leached+farmyard manure, and sawdust-leached+coconut coir pith showed similar and slightly higher pH values. The highest mean pH (7.81) was recorded in vermicompost-leached alone, followed by sawdust-leached and a combination of sawdust-leached+vermicompost leached.

Table 2: Analysis of chemical parameters of different casing substrates in the commercial trial

Casing medium	pH	Electrical conductivity (µS)
Sawdust- leached+farmyard manure (1:1)	6.76	620.00
Sawdust- leached+spent compost (1:1)	6.63	600.00
Sawdust- leached+coconut coir pith (1:1)	6.79	450.00
Sawdust- leached+vermicompost-leached (1:1)	7.39	703.00
Spent compost-2 years old	7.17	507.00
Farmyard manure-2 years old	7.17	590.00
Coconut coir pith	7.15	245.33
Vermicompost-leached	7.81	774.67
Sawdust-leached	7.52	693.00
Control (farmyard manure+local soil)	6.52	479.67
CD (*p*=0.05)	0.09	8.64
SE(d)	0.04	4.12

Regarding electrical conductivity, coconut coir pith exhibited the lowest mean value (245.33 µS), significantly lower than sawdust-leached+coconut coir pith and the control. The highest mean electrical conductivity (774.67 µS) was observed in vermicompost-leached, followed by the combination of sawdust-leached+vermicompost-leached and sawdust-leached alone. These findings provide insights into the pH and electrical conductivity dynamics of various casing substrates, crucial for optimizing conditions in *A.bisporus* production.The pH of casing materials significantly influences mushroom yield, with the optimal range suggested by Peyvast et al. (2011) being slightly alkaline at 7.2 to 8.2.

3.2. Changes in pH of casing substrates with the progression of crop

3.2.1. Preliminary trial

The pH trend during the cropping period was investigated across various casing combinations at different intervals (0, +7, +14, +21, +28, +35, +42, +49 and +56 days) from the date of casing with data presented in Tables 3 and 4. Table 3 indicates a gradual decrease in pH from the casing date to the end of cropping. Regardless of the days after casing, the

Table 3: Changes in pH of different casing media with the progression of crop

Casing medium	pH days after casing									Overall mean
	+0	+7	+14	+21	+28	+35	+42	+49	+56	
Sawdust- leached	7.29	7.24	7.19	7.14	7.09	7.01	6.93	6.87	6.81	7.06
Farmyard manure- 2 years old	7.14	7.08	7.01	6.96	6.88	6.82	6.74	6.79	6.63	6.89
Vermicompost- leached	7.53	7.41	7.32	7.27	7.20	7.09	6.97	6.95	6.85	7.18
Local soil	7.80	7.72	7.64	7.56	7.50	7.42	7.33	7.24	7.15	7.48
Coconut coir pith	6.73	6.68	6.62	6.57	6.49	6.42	6.37	6.24	6.17	6.48
Spent compost- 2 years old	6.45	6.38	6.25	6.19	6.11	6.08	6.05	6.03	6.00	6.17
Control (farmyard manure+local soil)	6.50	6.46	6.39	6.35	6.29	6.24	6.21	6.18	6.12	6.30
Overall mean	7.06	7.00	6.92	6.86	6.79	6.72	6.66	6.61	6.53	
Factors	CD (p=0.05)			SE						
Casing medium	0.02			0.01						
Interval	0.02			0.01						
Casing medium x Interval	0.06			0.03						

Table 4: Changes in pH of casing media in the commercial trial with the progression of crop

Casing medium	pH days after casing									Overall mean
	+0	+7	+14	+21	+28	+35	+42	+49	+56	
Sawdust- leached+farmyard manure (1:1)	6.76	6.71	6.61	6.55	6.49	6.41	6.32	6.23	6.13	6.47
Sawdust- leached+spent compost (1:1)	6.63	6.57	6.49	6.40	6.33	6.28	6.19	6.12	6.07	6.34
Sawdust- leached+coconut coir pith (1:1)	6.79	6.74	6.69	6.61	6.52	6.45	6.39	6.33	6.18	6.52
Sawdust- leached+vermicompost-leached (1:1)	7.39	7.31	7.25	7.21	7.12	7.07	6.96	6.89	6.82	7.11
Spent compost- 2 years old	7.17	7.04	6.98	6.69	6.50	6.42	6.37	6.22	6.15	6.61
Farmyard manure- 2 years old	7.17	7.11	7.02	6.97	6.89	6.85	6.77	6.70	6.64	6.90
Coconut coir pith	7.15	7.07	7.00	6.95	6.90	6.82	6.78	6.73	6.67	6.90
Vermicompost- leached	7.81	7.72	7.65	7.50	7.43	7.35	7.22	7.13	7.05	7.43
Sawdust- leached	7.52	7.42	7.38	7.31	7.23	7.13	7.06	6.94	6.80	7.20
Control (farmyard manure+local soil)	6.52	6.47	6.41	6.37	6.30	6.26	6.19	6.13	6.08	6.30
Overall mean	7.09	7.01	6.95	6.85	6.77	6.70	6.62	6.54	6.46	
Factors	CD (*p*=0.05)			SE						
Casing medium	0.02			0.01						
Interval	0.02			0.01						
Casing medium x Interval	0.05			0.02						

mean maximum pH was significantly highest in local soil (7.48), followed by vermicompost-leached alone (7.18) and sawdust-leached (7.06). Conversely, the significantly lowest mean pH (6.17) was observed in spent compost-2 years old, followed by control (6.30) and coconut coir pith (6.48), irrespective of the casing media and days after casing.

Across all casing media, the mean maximum pH (7.06) was recorded on the day of casing (0 day), decreasing significantly at each 7-day interval and reaching its minimum (6.53) on the last sampling day, i.e., +56 days after casing.

3.2.2. *Commercial trial*

There was a consistent decline in pH from casing to the end of cropping (Table 4). Regardless of the days post-casing, significantly the highest mean pH (7.43) was found in vermicompost-leached alone, followed by sawdust-leached alone (7.20) and sawdust-leached+vermicompost-leached (7.11; 1:1; v/v).

Conversely, the lowest mean pH (6.30) was observed in the control, followed by sawdust-leached+spent compost (6.34; 1:1; v/v), sawdust-leached+farmyard manure (6.47; 1:1; v/v), sawdust-leached+coconut coir pith (6.52; 1:1; v/v), and spent compost-2 years old alone (6.61; 1:1; v/v) among different casing media, irrespective of days post-casing. Across all casing media, the mean maximum pH (7.09) occurred on the day of casing (0 day), declining significantly every 7 days and reaching the minimum (6.46) on the last sampling day, i.e., +56 days after casing. Analyzing the interaction with crop progression, pH was significantly highest in vermicompost-leached alone (7.81) at casing (0 day), followed by 7 (7.72) and 14 (7.65) days after casing. The lowest pH (6.07) was recorded in sawdust-leached+spent compost (1:1; v/v) after 56 days, statistically similar to control (6.08) after 56 days. Other casing media displayed intermediate pH levels over various casing durations.

3.3. Changes in electrical conductivity of casing substrates with the progression of crop

3.3.1. Preliminary trial

Salt concentration, assessed through electrical conductivity, was monitored during cropping at various intervals (0, +7, +14, +21, +28, +35, +41, +49, and +56 days after casing).

Among the casing media, local soil exhibited the highest mean electrical conductivity (1150.04 µS), significantly followed by vermicompost-leached (1126.15 µS) and sawdust-leached (1041.15 µS).

In contrast, coconut coir pith had the lowest mean electrical conductivity (641.67 µS), followed by spent compost-2 years old (841.89 µS) and control (867.52 µS), irrespective of the days after casing (Table 5).

3.3.2. Commercial trial

Regardless of the days after casing, the mean electrical conductivity was highest in vermicompost-leached (1162.37 µS), followed by sawdust-leached+vermicompost-leached (1058.81 µS; 1:1; v/v) and sawdust-leached alone (1044.52 µS; 1:1; v/v).

On the other hand, the lowest mean electrical conductivity (658.56 µS) was observed in coconut coir pith alone, followed by sawdust-leached+coconut coir pith (735.30 µS; 1:1; v/v) and spent compost-2 years old (882.96 µS), statistically comparable to sawdust-leached+spent compost (885.70 µS; 1:1; v/v), irrespective of days after casing (Table 6).

Across all casing media, mean electrical conductivity was significantly lowest (566.27 µS) on the casing day (0 day), increasing significantly after each observation interval and reaching its peak (1285.50 µS) after 56 days.

Furthermore, coconut coir pith alone recorded the minimum salt concentration (245.33 µS) on the casing day (0 day), significantly followed by the same treatment after 7 days (339.00 µS) and sawdust-leached+coconut coir pith (450.00 µS; 1:1; v/v) on the casing day (0 day). Conversely, vermicompost-leached exhibited the maximum salt accumulation (1560.67 µS) after 56 days, significantly followed

Table 5: Changes in EC of different casing media with the progression of crop

Casing medium	EC days after casing									Overall mean
	+0	+7	+14	+21	+28	+35	+42	+49	+56	
Sawdust- leached	688.00	721.33	849.67	924.00	1,049.33	1,108.33	1,251.67	1,355.00	1,423.00	1041.15
Farmyard manure - 2 years old	593.67	624.00	705.67	784.00	867.33	995.00	1068.33	1,183.00	1,335.67	908.30
Vermicompost-leached	762.33	886.00	916.33	1,013.00	1,106.67	1,245.00	1,333.00	1,445.67	1,527.33	1126.15
Local soil	762.33	839.00	973.67	1043.67	1,110.00	1,246.33	1,389.33	1,410.67	1,575.33	1150.04
Coconut coir pith	238.00	321.00	425.00	560.33	623.33	742.00	851.33	956.33	1,057.00	641.67
Spent compost- 2 years old	498.67	553.00	613.67	764.33	809.67	916.67	1,074.00	1,103.00	1,244.00	841.89
Control (farmyard manure+local soil)	462.67	576.33	626.67	784.00	895.00	955.33	1,028.67	1,195.67	1,283.33	867.52
Overall mean	572.24	648.38	730.10	839.05	923.05	1,029.90	1,142.33	1,221.33	1349.38	
Factors	CD (p=0.05)			SE						
Casing medium	12.06			6.36						
Interval	14.30			7.22						
Casing medium× interval	37.82			19.09						

Table 6: Changes in EC of casing media in the commercial trial with the progression of crop

Casing medium	Electrical conductivity days after casing									Overall mean
	+0	+7	+14	+21	+28	+35	+42	+49	+56	
Sawdust- leached +farmyard manure (1:1)	620.00	700.67	822.67	970.00	1,053.67	1,108.00	1,178.67	1,202.67	1,294.67	994.53
Sawdust-leached+spent compost (1:1)	600.00	691.00	756.67	793.33	838.33	982.00	1017.67	1,112.00	1,180.33	885.70
Sawdust- leached +coconut coir pith (1:1)	450.00	538.67	593.33	641.00	708.67	792.33	873.00	962.67	1,058.00	735.30
Sawdust- leached +vermicompost-leached (1:1)	703.00	775.33	851.00	921.00	1,089.67	1,173.33	1,267.67	1,327.33	1,421.00	1058.81
Spent compost- 2 years old	507.00	637.33	725.67	764.33	897.67	925.67	1055.00	1199.00	1235.00	882.96
Farmyard manure- 2 years old	590.00	620.00	700.67	781.33	860.33	910.67	1065.67	1,188.00	1,323.33	893.33
Coconut coir pith	245.33	339.00	483.67	509.00	666.00	794.00	829.67	991.67	1,068.67	658.56
Vermicompost-leached	774.67	808.67	989.00	1,014.00	1,190.33	1,296.33	1,381.67	1,446.00	1,560.67	1162.37
Sawdust- leached	693.00	728.00	855.67	930.00	1,050.33	1,112.33	1,251.33	1,350.00	1,430.00	1044.52
Control (farmyard manure +local soil)	479.67	686.33	738.67	790.00	895.00	945.33	1,028.67	1,195.67	1,283.33	893.63
Overall mean	566.27	652.50	751.70	811.40	924.98	1,003.99	1,094.90	1,197.50	1,285.50	
Factors	CD (p=0.05)			SE						
Casing medium	4.54			2.30						
Interval	4.30			2.18						
Casing medium× Interval	13.61			6.89						

by the same material after 49 days (1446.00 µS) of casing. Intermediate salt levels were observed in other casing media over different observation durations. Chemical properties of casing media undergo changes during crop progression due to the accumulation of various ions and salts from mushroom mycelium metabolic activities. pH consistently decreased, and electrical conductivity increased across all tested casing media, either individually or in combinations. This aligns with findings by Jarial and Shandilya (2005a&b) and Szukacs and Geosel (2018), who also observed a declining pH and rising electrical conductivity during crop progression. The decrease in pH is attributed to mushroom mycelium releasing oxalic acid, while the increase in electrical conductivity is linked to elevated concentrations of ions such as calcium, potassium, sodium, chloride, and sulphate in the casing layer, contributing to heightened salinity (Shandilya, 1989).

4. Conclusion

pH and EC were assessed during the crop development stages of *Agaricus bisporus* across various casing substrates. pH ranged from 6.45 (spent compost) to 7.81 (vermicompost-leached), while EC varied from 238 µS (coconut coir pith) to 774.67 µS (vermicompost-leached). A consistent decrease in pH and increase in EC was observed up to 56 days after casing. These findings indicate that mushroom mycelium's metabolic activities significantly impact the chemical composition of the casing media, leading to pH reduction and EC elevation.

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